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THE FERTILIZATION OF ALBUGO CANDIDA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XIX.

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(WITH PLATE XXII)

THE recent studies of Mr. Stevens (99) upon *Albugo bliti* have yielded some exceedingly interesting results, which are very suggestive when considered and applied theoretically to a number of the Phycomycetes whose sexual processess are so little understood. Indeed, it is possible that the generally accepted views as to the homologies of sexual organs in certain members of this group may be materially changed with the newer standpoint.

It was my privilege to follow the investigation of Mr. Stevens throughout its progress. Many questions arose for future investigation to test the suggestive conditions presented by *Albugo bliti* in other Phycomycetes. It seemed also desirable to re-examine related species of Albugo, and Mr. Stevens very kindly gave me his collections of the oft-studied *A. candida*, which furnished the material for this study.

The results confirm the views of the earlier investigators, Wager (96) and Berlese (98), as to the behavior of the sexual nuclei. But a somewhat more detailed examination of a number of points in the cytology of the oogonium will, I trust, warrant the publication of this paper. The writer has also allowed himself to discuss the subject of fertilization in which multinucleate gametes are concerned, endeavoring to present a point of view that seems to him fruitful of future results. This portion of the paper is headed Theoretical Considerations.

The material of *A. candida* came from the vicinity of Columbus, Ohio, and had been fixed by three methods, in chrom-acetic acid, by the weak formula of Flemming, and in saturated solution of corrosive sublimate. That fixed in chrom-acetic acid proved very much the best for the present study, which desired the protoplasmic contents of the cells as free as possible from all inclusions such as oils and fats. It is interesting to know that the osmic acid of Flemming's fluid so preserved the oil-like material in the protoplasm as to render it insoluble in ordinary clearing agents, and very troublesome in all preparations, because the globules stained deeply and masked the protoplasmic elements. The material killed in corrosive sublimate did not present well-preserved nuclei or mitotic figures. The sections, cut 5μ thick, were stained with safranin, gentian-violet, and orange G.

The preparations confirmed in all essentials Wager's account of the development of the oogonium and antheridium.

A most interesting stage in this process is the differentiation of the so-called "receptive spot," a region of densely staining protoplasm in the oogonium at the point where the antheridium becomes applied to the structure. It is certainly true that a papilla from the oogonium is the active agent which works its way through the cellulose walls and establishes communication with the antheridium. In *Albugo bliti* the papilla actually pushes into the antheridium, becoming a swollen process in the interior of that structure. The papilla is much less pronounced in *A. candida*, but behaves in a strictly analogous fashion.

The antheridial tube begins its development and growth into the oogonium before there is the slighest trace of the oosphere, and it may have penetrated to one fifth the diameter of the structure before that cell is differentiated.

The development of the oosphere is an exceedingly interesting and complex process. Its commencement is indicated by a gradual accumulation of the protoplasm at the center of the oogonium. The nuclei, very variable in number (Wager estimates from 70–110), are at first in the resting condition, but very soon prepare for and pass through a mitosis. They lie in a coarsely vacuolate cytoplasm.

The first stage in the differentiation of the oosphere is illustrated in *fig. 1*, which shows a slight but not conspicuous increase in the granular protoplasm at the center of the oogonium. There soon appears, however, a remarkable structure in this region of denser protoplasm. It develops form and organization, and finally becomes a very prominent element in the oogonium. Mr. Stevens found a similar body in *A. bliti*, appearing at this period in ontogeny, and believing it to be associated with important activities in the oogonium called it the "coenocentrum."

The coenocentrum of *A. candida* is very much larger than that of *A. bliti*, and has attracted the attention of several observers. Wager was the first to recognize its protoplasmic nature, which appears unquestionable through its structure, development, and reaction to stains and clearing agents. When fully differentiated the coenocentrum is a sphere of dense, deeply-staining, slightly granular protoplasm, entirely free from inclusions, and two to four times the diameter of the nuclei. It is sometimes surrounded by a zone of lightly staining protoplasm, as is shown in *fig. 2*, through which delicate radiations may be traced. The coenocentrum develops, as has been said, from an accumulation of protoplasm in the center of the oogonium (*fig. 1*). It reaches its most beautiful state of differentiation when the appearance is that presented by *fig. 2*. Later the structure contracts somewhat, rounds itself off, and becomes a very dense body with a firm outline, as is indicated in *figs. 3–6*. It is then very conspicuous, staining deeply, but is not so plainly related to the cytoplasm as in earlier stages. Certain investigators have mistaken these later conditions for accumulations of oil or similar matter.

The differentiation of the oosphere, that is the separation of the ooplasm from the surrounding periplasm, is heralded by the appearance of the coenocentrum. The phenomenon is remarkable. It consists of an inward movement of the protoplasm toward the center of the oogonium, and a consequent floating out of the larger vacuoles to the periphery. The position of the nuclei is also affected so that they pass from the interior to variable distances between the coenocentrum and the wall of the oogonium. At a certain stage the nuclei may be found in a hollow sphere of protoplasm, at or just outside of the position where the wall of the oospore will finally be laid down. This is the very interesting and striking condition to which Mr. Stevens has applied the term "zonation."

Zonation is a very prominent stage of oogenesis in *A. candida*, although not as conspicuous as in *A. bliti*. It is similar in all important particulars, as may be seen by comparing *fig. 2* of this paper with *figs. 61, 62, 64, 65* of Mr. Stevens' article. The agreement is made more striking by the fact that in *A. candida* also the nuclei are usually in mitosis at this time. At the completion of zonation the ooplasm may be said to be differentiated from the periplasm, and only lacks the necessary female sexual nucleus to become the oosphere ready for fertilization.

At this point in oogenesis *A. candida* and *A. bliti* depart widely from one another. The oosphere of *A. candida* is uninucleate. One of the nuclei from a point near the periphery of the ooplasm slips back to the center and takes a position near the coenocentrum (*figs. 3, 4, 5*). One may find stages where such a solitary nucleus lies midway between the coenocentrum and the periplasm, but it is exceedingly difficult to determine in such cases whether the nucleus is returning to the center of the ooplasm or passing to the exterior. In the former case it would always be a resting nucleus, in the latter it might be in mitosis.

There can be no question but that the oosphere of *A. candida* is quite generally if not universally uninucleate. There are theoretical reasons why in the light of Mr. Steven's results with *A. bliti* one may expect some variation in this particular, and that *A. candida* may at least occasionally have a multinucleate oosphere. Indeed a few of the author's preparations look suspicious, but their rarity would make the establishment of such a point a very tiresome and laborious investigation, if possible at all.

It will be remembered that in *A. bliti* (according to Stevens' paper) a large number of nuclei pass into the ooplasm when the oosphere is differentiated, and these are doubled in number by a mitosis, so that the oosphere when ready for fertilization contains about one hundred nuclei, and may be described as a compound oosphere. Fertilization is effected by the introduction of a large number of sperm nuclei (about one hundred) from the antheridial tube, and these, after becoming distributed through the ooplasm, finally fuse in pairs with the female nuclei.

It is very surprising that two species of the same genus, organisms so closely related as are *A. candida* and *A. bliti*, should present such a remarkable distinction as regards processes of fertilization. It was this that led the author to the examination here described. But *A. candida* certainly presents, as all investigators agree, the characteristics of the usual method of fertilization, when one male nucleus fuses with one female in an oosphere. If there is any variation in this particular it has not been demonstrated, and is probably very rare, although theoretically possible. It is to be hoped that some material of this or other species will be found to bridge over the gap between the conditions illustrated by *A. candida* and *A. bliti*.

Passing on for the present to the consideration of the antheridial tube, there is nothing to be said except in confirmation of previously published accounts. The tube at the time of zonation (*fig. 2*) is very near if not directly applied to the ooplasm. It rapidly elongates and increases in size until there is presented the club-shaped structure shown in *fig. 3*, containing granular protoplasm. A nucleus, the sperm, very soon slips from the antheridium and occupies a position in the dense cytoplasm near the tip of the tube, as is shown in *figs. 3* and *4*. The delicate wall around the tip of the antheridium finally dissolves, and the sperm nucleus is introduced into the ooplasm, at first surrounded by a quantity of granular cytoplasm from the antheridium (*fig. 5*), which immediately mingles with that of the oogonium.

Although there is no proof that more than one sperm nucleus ever enters the oosphere, it is well to note that other nuclei may be present in the upper portion of the antheridial tube. Such an example is shown in *fig. 3*, where one perfectly organized nucleus occupies the neck of the tube. It would not seem strange to the writer if material were sometimes found where several nuclei occasionally or frequently occupied the tip of the antheridial tube, and were introduced into the ooplasm at the time of fertilization.

The sperm nucleus begins to move towards the center of the oosphere, and finally fuses with the female nucleus near if not actually adjacent to the coenocentrum. *Figs. 6* and *7* show the two sexual nuclei close to one another, and *fig. 8* is of a fusion nucleus.

An important change usually comes over the coenocentrum before or during the fusion of the sexual nuclei. The body so clearly differentiated at the time when the antheridial tube first enters the ooplasm becomes less and less clearly defined, and at last completely disappears.

The dissolution of the coenocentrum may be accomplished in two ways. The structure may increase in size, the outline becoming vague, until there is finally present an irregular mass of protoplasm usually surrounding the fusion nucleus. Or the coenocentrum may fragment into several portions, as is shown in *fig. 7*, and these later swell and merge into an unorganized granular protoplasmic mass.

It seems quite certain that the coenocentrum is not a permanent structure in the protoplasm. Arising as an accumulation of protoplasm at the center of the oogonium, the coenocentrum becomes most pronounced when the oosphere is organized, but finally all trace of the body is lost in the ripening oospore.

The suggestion of Swingle (98) that the coenocentrum is an organ of the oosphere seems less probable when we consider its temporary character. Yet it is possible that the coenocentrum may have important functions to perform during oogenesis. The differentiation of the ooplasm and the separation of the female nucleus from all the other nuclei of the oogonium which pass into the periplasm is a remarkable phenomenon. However, there is little or no evidence furnished by morphology as to the part which the coenocentrum might play in these activities. On the other hand, there is the possibility that the structure may represent only an effect of functions concerned with the protoplasm of the oogonium as a whole. It may be the morphological expression of dynamic activities deeply seated in the protoplasm, such as might be presumed to operate when the ooplasm collects in the center of the oogonium, when the nuclei pass outward into the periplasm, and when the female nucleus is chosen to preside over the oosphere. This point of view is worth considering, although we have not yet enough data to safely advance such a theory.

The nuclei of *A. candida* are so small that the study of the mitotic figure is very difficult. Stages of division are always numerous in preparations, because both the antheridium and oogonium present a mitosis at one stage of their development, apparently affecting all of the nuclei simultaneously. The mitosis in the oogonium takes place when the ooplasm is differentiated from the periplasm ; that in the antheridium before the penetrating tube has entered the ooplasm. Metaphase is a very conspicuous stage. As is shown in *fig. 10* the spindle is entirely intranuclear. The writer was not able to establish with certainty the presence of centrosomes, but the nuclear figure is very small, and proportionally a centrosome might be expected to be exceedingly minute. The chromosomes are granular, and the number can only be estimated with great difficulty, but Wager's view that there are about 12 or 16 is probably correct. They arise from a linin network that may be readily stained in the resting nucleus (*fig. 9*). The nuclear membrane persists until late anaphase, as is illustrated in *fig. 11*, where the two sets of daughter chromosomes are shown separated and massed at the poles of the elongated nucleus. The old nuclear membrane finally dissolves, leaving the two groups of daughter chromosomes quite apart (*fig. 12*), after which each daughter nucleus proceeds to organize its new membrane. The intervening space, at first vacuolate, becomes filled with granular cytoplasm and the mitosis is finished.

There appears to be no positive evidence that the mitoses described above for the oogonium and antheridium are reducing divisions, whatever may be the speculations upon that point. Little can be said on this subject in our present very incomplete knowledge of the mitotic figures at other periods in the life history of Albugo. The study of the oospore may lead to some important results, although the writer's incomplete observations were no more promising than the examinations of Wager (96), Berlese (98), and Stevens (99). An investigation of this subject is much to be desired, and should include the study of the nuclear figure at some period of vegetative activity. *A. candida*, however, is not the most favorable subject for such an examination, as the nuclei are small. *A. portulacae* or *A. bliti* would be more satisfactory.

THEORETICAL CONSIDERATIONS.

The writer must confess a feeling of considerable uncertainty as to the cytological conditions and perhaps homologies of the sexual organs found in certain Phycomycetes. Much interesting material is likely to be presented through future investigations in this field, and particularly upon those forms whose gametes are well known to be multinucleate. Accordingly any review of the present situation can only offer suggestions of what may later become established by research. The subject is a very interesting one, but the difficulties of investigation are unusual, not only on account of the necessary technique, but in the nature of the material that must be studied.

It is well known that the gametes of certain fungi contained in the Mucorales are multinucleate. Several investigators have studied these structures, and the zygospore which results from their fusion, but none have given a clear account of the cytological phenomenon exhibited in this act of fertilization. The descriptions of Léger (95) and Dangeard and Léger (94) present some interesting conditions, which, however, are very difficult to understand in the light of generally accepted theories of fertilization, and certainly are not at present thoroughly explained.

When the term gametes was first applied to the fusing cells that formed the zygospore of a mould, it was probably in the belief that these were sexual elements pure and simple, homologous with other sexual cells, such as the oospheres and sperms of the algæ, the fusing cells in forms of the Conjugatae, or the members of a copulating pair of swarm spores. The term gamete had a strict morphological significance implying general homologies, in most cases very close, in other instances more remote, or possibly only a relationship through some earlier non-sexual form of spore.

At present the gamete probably stands in the minds of most botanists as a morphological unit, with a structure essentially the same in all realms of biology. The gamete is a uninucleate sexual body, with a greater or less amount of cytoplasm which may be very much specialized, as is illustrated in the degree of differentiation shown by the egg and the sperm.

The question that must suggest itself to many is the correctness of the use of the term gamete, with its implied homologies, when designating the fusing sexual elements in a number of Phycomycetes. To state the difficulty concretely: are the multinucleate cells that fuse to form the zygospore of the Mucorales, the antheridial tubes and oospheres of the Peronsporales and Saprolegniales, homologous with the simpler sexual elements of the Monoblepharales and Chytridiales, and the gametes of the algæ? Although we know practically nothing about the cytology of these last two groups of fungi, nevertheless the studies of Thaxter (95) on Monoblepharis and the structure of the Chytridiales indicate much simpler conditions than appear in the Mucorales, Peronosporales, and Saprolegniales. They probably agree in all essentials with the sexual processes of the algæ.

In the Mucorales all that we can safely affirm now is the fusion of two multinucleate masses of protoplasm. The history and fate of the nuclei is certainly not clearly understood, but the indications are rather for some complex phenomenon involving many nuclei than a simple act of fertilization, concerned with only two sexual nuclei.

The Peronosporales present two well defined conditions at the time of fertilization. The phenomenon in *A. bliti* is at variance with that established for *A. candida*, and indicated by superficial examination for several other forms. In *A. candida* the sexual act is the fusion of two nuclei, thus satisfying the requirements generally understood by fertilization. In *A. bliti* there are many sexual nuclei fusing in pairs, about 100 sperm nuclei entering a compound oosphere which contains approximately 100 female nuclei.

What are the homologies between these two species? The oogonia and antheridia are certainly homologous structures, and there is no evidence that the nuclei contained within are not likewise. There seems indeed, from the cytological data at hand, good reason to believe that these nuclei are all homologous, both those that fuse and those that remain sexually inactive, either left behind in the antheridium or lying in the periplasm of the oogonium. Our inability at present to distinguish between the various mitoses in either sexual organ offers the strongest evidence of the position above taken. The difference between these two species is perhaps then only one of the number of the nuclei that are actually sexual or gametes in the strictest morphological sense. Nuclei that are not actually sexual, either in the antheridium or periplasm of the oogonium, may stand phylogenetically for a previous condition, when these organs contained a much larger number of sexual nuclei. This point of view seems to have been in the mind of Hartog (91).

The antheridium and oogonium of Albugo are gametangia. However remarkable is the difference between *A. candida* and *A. bliti* it is nevertheless merely a question of the number of nuclei that become sexually functional or active gametes. In

A. bliti there are many such; in *A. candida* the number becomes reduced to two.

Although the oosphere of *Albugo bliti* is in one sense a gamete, we should be careful to note the peculiarities that make such a designation loose and undesirable. In reality, this structure, which was called a compound oosphere by Mr. Stevens (**99**), is made up of many gametes (sexual nuclei) acting cooperatively in a common mass of cytoplasm. It may appear desirable to some to give a name to such a structure, one that shall express the idea of sexuality, although it may modify the usual conception of a gamete. The writer suggests the term *coenogamete* as applicable to a multinucleate mass of protoplasm, whose individual nuclei are sexual elements.

Let us think of the sexual processes of the mould in this light. The two elements that unite to form the zygospore are multinucleate. It is possible that some or many of these nuclei are sexual in function (this awaits investigation), and if this be true the fusing masses of protoplasm will illustrate admirably the writer's idea of coenogametes. The sexual nuclei are gametes in the strict sense of the word, and the cells that contain them are gametangia.

It seems to the writer that a standpoint is presented which may be of very great service in the investigations that will be necessary to clear up the peculiar difficulties of this field of research. Although the subject must be approached almost entirely theoretically and tentatively, he ventures to suggest some possibilities to await the verdict of future studies.

Is it necessary that the oospheres of the Peronosporales and Saprolegniales be regarded as homologous with similar structures in the algæ? It is conceivable that the degree of differentiation illustrated by the oosphere may have arisen entirely independently of the algæ, after the fungal ancestors of these groups had departed from the chlorophyll-bearing thallophytes.

Let us suppose a group of fungi with gametangia that discharge motile gametes which fuse in pairs in the water after the manner of the swarm spores of many algæ. Such forms might readily enough have come from several groups of algæ. Let us again suppose such types of fungi to leave the water and live a terrestrial life as parasites or saprophytes. Their gametangia, unable to discharge the gametes into an aqueous medium, and yet required to satisfy the chemotactic influences associated with sexuality, might find the most satisfactory escape from the difficulties of their environment in fusing with one another. In such mingling of the cytoplasm of two gametangia would be presented the possibility of the fusion in pairs of many of the sexual nuclei (gametes) in a protoplasmic medium instead of water. From such conditions might have arisen a zygospore similar to that of the Mucorales.

If a method of zygospore formation should develop in the manner indicated above, what further changes might be expected with the gradual evolution and specialization of the forms? It does not seem unreasonable to suppose that the same tendencies toward the differentiation of sex would appear here as are manifested in all groups of the algæ. It might be advantageous to reduce the number of gametes (sexual nuclei) to provide the functional female ones with a special supply of cytoplasm, and thus to organize one or more oospheres inside a female gametangium. The furthest extreme of such sexual differentiation would be similar to the conditions illustrated by *Albugo candida*, with its single functional female nucleus (gamete), associated with a large supply of cytoplasm (ooplasm). We have in *Albugo bliti* an example of what might be imagined as an earlier stage in such a process of differentiation, for the number of functional sexual nuclei or gametes is large. Nevertheless, the female gametes are collected in a differentiated region of the cytoplasm, the oosphere, and this peculiarity would be a decided advance over the conditions illustrated by the moulds.

It should be noted that the oospores resulting from this differentiation of sex would bear no homologies to analogous structures among the algæ. They would furnish, on the contrary, another illustration of the well established fact that many divergent lines of thallophytes have developed, independently of one another, the oosporic method or sexual reproduction

The bearing of such possibilities as have been considered above upon the phylogeny of the Phycomycetes is of course attractive, but hardly within the range of the present discussion

It may be questioned whether the theoretical consideration of and speculation upon such difficult subjects as these we have so briefly outlined is worth while. Certainly we have few or no facts to warrant the expression of positive opinions, yet attempts to understand clearly and explain even the most obscure subjects may suggest methods of approach to others working for the same ends, and such hopes must be their justification.

SUMMARY.

Communication between the oogonium and antheridium is established by a papilla from the former structure, which works its way through the cellulose walls into the antheridium.

The differentiation of the ooplasm is associated with the appearance of an organized spherical protoplasmic body in the center of the oogonium. This structure has been named the coenocentrum (Stevens, **99**).

A conspicuous stage in oogenesis is that called zonation. Then the nuclei, usually in mitosis, lie at or near the inner boundary of the periplasm, and the coenocentrum is very prominent in the center of the ooplasm.

The oosphere is organized after the stage of zonation, when one of the nuclei from near the periphery returns to the interior of the ooplasm, and takes a position close to the coenocentrum. There is one mitosis in the oogonium, which occurs usually at or slightly before the time of zonation. There is no proof that this mitosis is a reducing division. The spindle is intranuclear, and the nuclear membrane persists until late anaphase. It was not possible to establish the presence of centrosomes, possibly because the mitotic figure is very small. The number of chromosomes is probably 12 to 16 (Wager's estimate).

The oosphere of *A. candida* is certainly generally, if not universally, uninucleate. This point was studied with especial care, in view of the conditions described by Mr. Stevens (**99**) in *A. bliti.*

The tip of the antheridial tube at the time of zonation is very near if not directly applied to the ooplasm. As it penetrates the oosphere a nucleus slips down into the swollen end whose surrounding wall is later dissolved, and the sperm nucleus is then introduced into the ooplasm surrounded by a quantity of dense cytoplasm.

The sperm nucleus approaches the female nucleus and slowly fuses with it in close proximity to the coenocentrum.

The coenocentrum begins to disorganize at about the time of the fusion of the sexual nuclei. The dissolution may be accompanied by fragmentation, but the final result is always the same, namely an irregular ill-defined region of dense protoplasm in the oospore that finally becomes indistinguishable from the surrounding cytoplasm.

The coenocentrum is not a permanent structure in the protoplasm. Arising as an accumulation of cytoplasm, it becomes most conspicuous at about the time of zonation, finally disappearing after the fusion of the sexual nuclei. The writer suggests that it may be merely the morphological expression of the remarkable activities displayed by the protoplasm, of the oogonium as a whole at the time of the differentiation of the oosphere.

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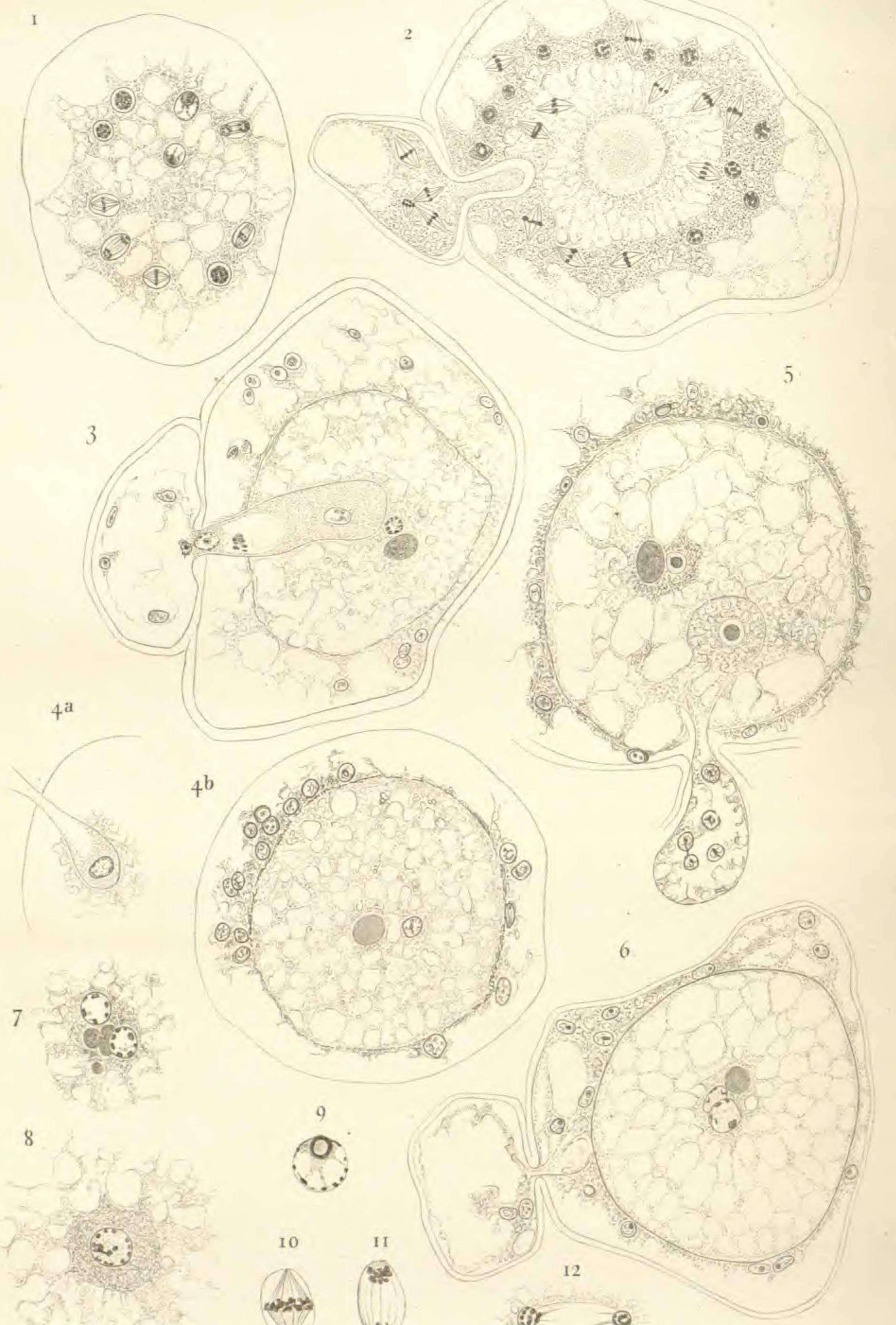

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DAVIS on ALBUGO.